THE EXPLOSION OF THE SPERMATOZOA OF THE CRAB *LOPHOPANOPEUS BELLUS* (STIMPSON) RATHBUN.

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(FORTY-SIX FIGURES.)

CONTENTS.

INTRODUCTION.

For a number of years the writer has been studying the male germ cells of the Decapoda with two purposes in mind: (1) to discover the means by which the mature, dormant spermatozoa of the Decapoda become activated, in order to shed light on the problem of fertilization in this order of Crustacea, and (2) to trace more clearly the process of spermatogenesis. The present paper on the explosion of the spermatozoa of the black-clawed crab, *Lophopanopeus bellus*, is a contribution involving the former of these problems.

MATERIAL AND METHODS.

The material for this study consisted of the living spermatozoa of *Lophopanopeus bellus*, common in certain localities around the Puget Sound Biological Station, Friday Harbor, Wash. The spermatozoa of this crab are very favorable for study in that they are not enclosed by the numerous spermatophores so common in other brachyura. As pointed out in another paper (Fasten, 1917), "in *Lophopanopeus bellus* it doesn't seem as if numerous spermatophores are developed. Here it appears that one large spermatophore is formed in which the spermatozoa are tightly packed." Since this is the condition all that was necessary to

obtain a plentiful supply of living spermatozoa was to rupture the deferent ducts and the male gametes oozed out in tremendous numbers.

The living spermatozoa were studied in the same manner as described in my earlier paper on the spermatogenesis of the edible crab, *Cancer magister* Dana (Fasten, 1918). Numerous spermatozoa suspended in the crab's body fluid, or in sea water which is isotonic with the crab's body fluid, were placed on a slide and covered with a cover glass. These could then be studied with the high power oil-immersion lenses. By allowing various chemical solutions to diffuse under the cover glass all changes in the spermatozoa could be observed and outlined with the aid of the camera lucida.

The living spermatozoa were studied in the following solutions:

1. Crab's body fluid.
2. Sea water.
3. Sodium chloride (NaCl)—M/2 NaCl and less.
4. Sodium nitrate ($NaNO_3$)—M/2 $NaNO_3$ and less.
5. Calcium chloride ($CaCl_2$)—3/8M $CaCl_2$ and less.
6. Potassium chloride (KCl)—M/2 KCl and less.
7. Potassium nitrate (KNO_3)—M/2 KNO_3 and less.
8. Potassium hydroxide (KOH)—very dilute solution.
9. Distilled water.
10. Cane sugar ($C_{12}H_{22}O_{11}$)—M/1 $C_{12}H_{22}O_{11}$.
11. Ovarian fluid.
12. Acidulated sea water. Various small amounts of acids were added to sea water, such as: glacial acetic, salicylic, saponin, sodium glycocholate, nitric, hydrochloric, oxalic, tannic, picric, and chromic acids.

Many of the spermatozoa in all stages of explosion were fixed on the slide with either osmic acid fumes, or Bouin's fluid, or Flemming's mixture, and then stained with Heidenhain's iron-hæmatoxylin and acid-fuchsin. Those fixed with osmic acid fumes gave beautiful results, so that the stained elements were perfect representations of the living structures. This can be clearly seen when one examines Figs. 3–7, which are from stained preparations fixed with osmic acid fumes, and compares them with figures 1 and 2 which are from living spermatozoa suspended in the body fluid of the crab.

Normal Appearance of Spermatozoa.

The living spermatozoa of *Lophopanopeus bellus* when studied in the cœlomic fluids of the crab are found to be small, greenish bodies, which appear like spheroids when seen from the top or bottom (Fig. 1), and like ellipsoids when viewed from the side (Fig. 2). In structure they seem to be similar to those of *Cancer magister*. Within the centre there is a clear central body (Figs. 1 and 2, *b*) and surrounding this are two vesicles; a uniform, darkly green secondary vesicle (Figs. 1 and 2, *v'*), and a clear, transparent primary vesicle (Figs. 1 and 2, *v*). Outside of these vesicles is a granular and vacuolated protoplasmic cup (Figs. 1 and 2, *h*) of a lighter greenish hue than the secondary vesicle. If the spermatozoa remain suspended in the crab's body fluids for some time their protoplasmic cups open up and liberate the radial arms (Figs. 3–7). It is thus seen that the protoplasmic cup of the spermatozoön consists of a nuclear cup (Fig. 3, *n*) and radiating radial arms (Fig. 3, *r*).

When the spermatozoa are fixed with osmic acid fumes and stained by the iron-hæmatoxylin and acid-fuchsin methods, then the nuclear cup, radial arms and the central body stain black (see Figs. 3–7), the second vesicle stains a dark amber, whereas the primary vesicle remains transparent.

Four types of spermatozoa are produced, depending on their number of rays. There is a three (Fig. 4), four (Fig. 5), five (Fig. 6), and a six (Fig. 7) rayed type. The four (Fig. 5) and five (Fig. 6) rayed types, however, are produced in largest numbers. These rays are not pseudopodia-like processes similar to those which Binford ('13) pictures for the spermatozoa of *Menippe mercenaria*. They are distinct arms similar to those found in the crayfish *Cambarus virilis* and *Cambarus immunis*, as pictured by the writer in a previous paper on the spermatogenesis of these forms (Fasten, 1914).

Effects of Chemical Agents on Spermatozoa.

1. *Sea Water.*—Sea water produces no change in the normal appearance of the spermatozoa. The protoplasmic cup, however, swells slightly and liberates the radial arms (Figs. 8 and 9).

2. *Sodium Chloride.*—An M/2 NaCl solution which is isotonic

with sea water produces no change in the normal appearance of the spermatozoa (Figs. 10 and 11). An M/4 NaCl solution brings about a slight shrinkage in the nuclear cup, otherwise there is no further change. In an M/6 NaCl solution the secondary vesicle is very slowly everted. First of all it squeezes out in the form of a small bubble (Fig. 12), until very gradually it assumes the appearance shown in Fig. 13. In an M/7 NaCl solution the eversion of the secondary vesicle is much faster. Figs. 14, 15 and 16 show successive stages in the eversion process. Most of the spermatozoa proceed to the stage shown in Fig. 16 and then cease. An M/8 NaCl solution brings about a complete and rapid explosion of all the spermatozoa. Figs. 17, 18 and 19 show respectively the beginning, middle and end of the process. In Fig. 19 the secondary and primary vesicles, as well as the central body are seen completely everted.

3. *Sodium Nitrate.*—An M/2 $NaNO_3$ solution which is isotonic with sea water brings about no appreciable change in the normal appearance of the spermatozoa. In an M/4 $NaNO_3$ solution the only change noticed in the spermatozoa is a slight swelling of the nuclear cup. An M/8 $NaNO_3$ solution causes a slow eversion of the secondary vesicle, producing figures similar to those shown in Figs. 15 and 16. In an M/16 $NaNO_3$ solution the eversion of the two vesicles occurs rapidly and with considerable force, so that all the spermatozoa soon take on the appearance shown in Fig. 19.

4. *Calcium Chloride.*—A 3/8M $CaCl_2$ solution is isotonic with sea water and this brings about no change in the normal spermatozoa. A 3/11M $CaCl_2$ solution brings forth a partial eversion of the secondary vesicle (Fig. 20). In a 3/16M $CaCl_2$ solution the spermatozoa explode completely. The vesicles are entirely everted and at the same time the nuclear cup shrinks considerably and becomes irregular. Figs. 21–24 show various stages in the explosion process. In the $CaCl_2$ solutions the detailed structure of the spermatozoa can be clearly distinguished.

5. *Potassium Chloride.*—In an M/2 KCl solution which is isotonic with sea water the spermatozoa remain normal. In M/4 and M/8 solutions of KCl the only perceptible change produced in the spermatozoa is a disappearance of the granules

and vacuoles in the nuclear cup making it become more homogeneously green. Also the secondary vesicle shrinks somewhat, thereby leaving the clear primary vesicle to show more prominently (Fig. 25). An M/16 KCl solution produces swelling and explosion of the spermatozoa (Fig. 26). In many instances the explosion is so violent that the nuclear cup ruptures completely.

6. *Potassium Nitrate.*—An M/2 KNO_3 solution which is isotonic with sea water does not produce any explosion. However, the protoplasmic cup swells and becomes more homogeneous in appearance. Also the primary and secondary vesicles become more distinctly marked off from each other (Fig. 27). An M/4 KNO_3 mixture has a similar effect. An M/8 KNO_3 solution brings about a swelling of the protoplasmic cup and a slow eversion of the second vesicle so that the spermatozoa resemble Fig. 28. In an M/16 KNO_3 solution the spermatozoa explode very rapidly and they come to look like Fig. 26.

7. *Potassium Hydroxide.*—Very dilute solutions of KOH bring forth a violent reaction in the spermatozoa. The protoplasmic cup swells, becomes homogeneous and at the same time pushes the vesicles upward (Figs. 29–31). The secondary vesicle undergoes a rotation and is pushed to one side. Finally the vesicles explode with great violence and the entire spermatozoön soon goes to pieces.

8. *Distilled Water.*—Distilled water produces a rapid eversion of the vesicles so that in a very short time the spermatozoa come to resemble Figs. 32 and 33.

9. *Cane Sugar.*—From the above experiments two conclusions might be inferred regarding the explosion of the spermatozoa, one is that it is due to lack of electrolytes, and the other is that the explosion is due to a reduction of the osmotic pressure produced by surrounding the spermatozoa with a hypotonic solution. In order to determine which factor we have to deal with, the spermatozoa were surrounded with an M/1 cane sugar solution which is approximately isotonic with sea water. If the factor involved were due to lack of electrolytes then, since the sugar solution contains no electrolytes, the spermatozoa ought to explode. But the M/1 cane sugar solution did not produce any

change in the normal appearance of the spermatozoa, thereby pointing to the second factor, namely, osmotic pressure, as the one which undoubtedly operates in bringing about the eversion of the vesicles.

10. *Ovarian Fluid.*—Since a reduction in osmotic pressure produces the explosion of the spermatozoa, the next question which naturally arises is whether the female gonads at the time of fertilization produce a hypotonic substance which, when coming in contact with the spermatozoa, causes them to explode, thereby bringing about fertilization of the ova. In order to test this out, the ovaries and oviducts were mashed up in sea water and the living spermatozoa were then surrounded by this mixture. In some cases (not all), a few of the spermatozoa exploded violently. The nuclear cup at first swelled and became homogeneous (Fig. 34). Then the vesicles were everted with considerable force and in many instances, the primary vesicle, or both the primary and secondary vesicles completely disintegrated, leaving stages like those shown in Figs. 35–39. Whether this was due to some agent produced by the female gonads or to some other agent cannot be definitely stated, for not all of the spermatozoa were affected in the same manner as those mentioned above. However, it is also significant that the ovaries used during the months of the year when the investigations were conducted (June and July), were past maturity. They were small and immature and this might account for the results obtained. Another significant fact to be taken into consideration is that in control experiments in which living spermatozoa from the same males as those used in the experiments with the ovarian fluids, were surrounded with sea water alone, none of the spermatozoa exploded. Now, the question arises, why should we get a violent explosion of even a few spermatozoa when ovarian contents are used and no explosion when the ovarian fluids are lacking? I am strongly of the opinion that the female gonads produce some substance which is responsible for the explosion. Also, it seems very probable that at the time of sexual maturity of the female this specific substance must be present in such quantities as to activate all of the living spermatozoa.

11. *Acidulated Sea Water.*—In all cases weak dilutions of the

acids were used. If the acid was a liquid, the dilution used was 1 part of the concentrated acid dissolved in 25 parts of sea water. A drop of this was then added to the edge of the cover glass under which the living spermatozoa were held suspended in sea water. If the acid used was crystalline in texture, then a few of the crystals were placed at the edge of the cover glass and allowed to dissolve slowly under it.

(*a*) *Glacial Acetic Acid.*—Causes the protoplasmic cups to lose their granular and vacuolated appearance. Usually two or three dark granules remain in the nuclear cup. The nuclear cups and radial arms swell and lose their color (Figs. 40 and 41). The spermatozoa in many instances are thrown together into aggregates (Fig. 42). After remaining exposed to the action of the acid for some time many of the spermatozoa explode (Fig. 43) and disintegrate completely.

(*b*) *Salicylic Acid.*—Reaction here is similar to that caused by glacial acetic acid.

(*c*) *Saponin.*—Causes considerable swelling (Fig. 44). Nuclear cup and radial arms become more homogeneous and much paler in color. They appear almost transparent. A few of the spermatozoa explode after being exposed for some time.

(*d*) *Sodium Glycocholate.*—Causes swelling similar to that produced by saponin or glacial acetic acid. During this swelling the vacuoles of the nuclear cup at first enlarge and then disappear, giving the nuclear cup a homogeneous appearance. Soon a violent explosion of vesicles takes place. Nuclear cup now loses its greenish color, becomes ragged and transparent with small dark spots. Very shortly the spermatozoa disintegrate.

(*e*) *Nitric Acid.*—This brings about a homogeneity of appearance in protoplasmic cup with considerable shrinkage (Fig. 45). The second vesicle in many cases is everted (Fig. 46).

(*f*) *Hydrochloric Acid.*—The reaction here is very similar to that caused by nitric acid.

(*g*) *Oxalic Acid.*—Reaction is similar to that produced by nitric acid.

(*h*) *Tannic Acid.*—Reaction is similar to that of nitric acid, with the exception that none of the vesicles are everted.

(*i*) *Picric Acid.*—The reaction produced in the spermatozoa

is the same as that brought about by tannic acid. The spermatozoa are soon killed and stained a yellowish-green.

(*j*) *Chromic Acid.*—This produces a similar result to that obtained with either tannic or picric acids. Here the spermatozoa are fixed a yellowish-brown.

Discussion.

A careful examination of the data presented in this paper shows quite clearly that a lowering of the osmotic pressure in the medium which surrounds the spermatozoa is responsible for their explosion. In this respect the present research bears out what Koltzoff ('06) first suggested for the explosion of the spermatozoa of other decapods. Also, Binford ('13) in *Menippe mercenaria* and the present writer in *Cambarus virilis* (Fasten, '14), and *Cancer magister* (Fasten, '18), have found that osmotic pressure accounts for the explosion of the spermatozoa. In the light of all this accumulated evidence it seems quite certain that the stimulating agent which brings about the explosion in the spermatozoa of the Decapoda, is one which reduces the osmotic pressure in the medium that surrounds them.

Since this is the operating factor, the question which naturally suggests itself is where in the Decapoda is such a stimulating agent produced? The writer is strongly of the opinion that the mature gonads of the female decapod produce some chemical substance which, when it comes in contact with the spermatozoa, brings about their explosion. The experiments with the ovarian fluids seem to point to such a conclusion. The work of Koltzoff ('06) and Binford ('13) also suggests a similar conclusion.

Concerning the function of the explosion, it, undoubtedly, acts as the force or the motive power which drives the spermatozoön into the egg during the process of fertilization. What parts of the spermatozoön actually penetrate the ovum during fertilization is still a debated question. Koltzoff ('06) and Spitschakoff ('09) are in agreement that the nuclear cup (derived from the nucleus of the spermatid) is the only structure which enters the ovum. Binford ('13), on the other hand, claims that the everted vesicles (cytoplasmic structures) of the exploded spermatozoön are driven into the egg, whereas the nuclear cup

remains on the outside where it soon disintegrates. In order to bring this mode of fertilization in harmony with the idea of the continuity of the chromosomes, Binford suggests that "the contents of the capsule (vesicles) may be derived from the nucleus of the spermatid and is probably oxychromatin which deposits basichromatin after it enters the egg and so gives rise to the chromosomes in the male pronucleus."

It is thus obvious that more research along this line is essential before any definite conclusions regarding fertilization in the Decapoda can be formulated. If we accept Binford's results then we must admit that they are contrary to everything that we know regarding fertilization in animals.

Summary.

1. The spermatozoa of the black-clawed crab, *Lophopanopeus bellus* (Stimpson) Rathbun, are minute, greenish cells, which appear like spheroids when seen from the top or bottom and like ellipsoids when seen from the side.

2. The structure of these spermatozoa is very similar to that of the edible crab, *Cancer magister* Dana. In the centre there is a tube-like central body, and surrounding this in order of sequence is a secondary vesicle, a primary vesicle and a nuclear cup with slender radiating arms.

3. There are four types of spermatozoa produced in *Lophopanopeus bellus*, depending on the number of radial arms which they contain. There are three-, four-, five- and six-rayed spermatozoa, with the four- and five-rayed types predominating in numbers.

4. In sea water and isotonic solutions of various salts, no change occurs in the normal appearance of the spermatozoa. In hypotonic solutions of these salts the spermatozoa explode by an eversion of the two vesicles and the central body.

5. In ovarian fluids some of the spermatozoa explode violently, with a rupture and disintegration of one or both vesicles.

6. Acidulated sea water has a harmful effect on the spermatozoa, either causing swelling or shrinkage, with subsequent disintegration.

7. A lowering of the osmotic pressure in the medium that

surrounds the spermatozoa, undoubtedly brings about their explosion.

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DESCRIPTION OF PLATES.

All figures in the accompanying plates were made with the aid of the camera lucida. All figures, except Figs. 3–7, were made from living spermatozoa. Figs 3–7 are drawings of spermatozoa which were fixed by osmic acid fumes and stained with Heidenhain's iron-hæmatoxylin and acid-fuchsin. The magnification of Figs. 1–7 is 3,300 times; that of Figs. 8–33 is 1,400 times, and that of Figs. 34–46. is 1,700 times.

EXPLANATION OF PLATE I.

FIGS. 1 and 2. Bottom and side views of living spermatozoa suspended in crab's body fluid. *b*, central body; *h*, protoplasmic cup; *v*, primary vesicle; *v'*, secondary vesicle.

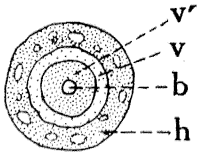
FIG. 3. Side view of spermatozoön fixed in osmic acid fumes and stained with iron-hæmatoxylin and acid-fuchsin. *b*, central body; *n*, nuclear cup; *r*, radial arms; *v*, primary vesicle; *v'*, secondary vesicle.

FIGS. 4 to 7. Bottom views of spermatozoa fixed in osmic acid fumes and stained with iron-hæmatoxylin and acid-fuchsin, showing, respectively, three-, four-, five- and six-rayed types.

FIGS. 8 and 9. Spermatozoa in sea water.

FIGS. 10 to 19. Spermatozoa in various concentrations of sodium chloride.

FIGS. 20 to 23. Spermatozoa in various concentrations of calcium chloride.



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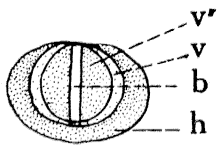

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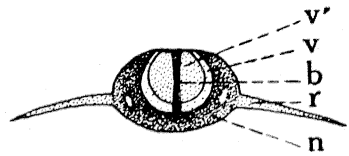

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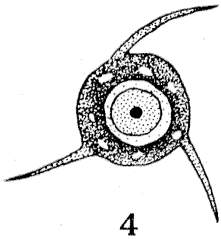

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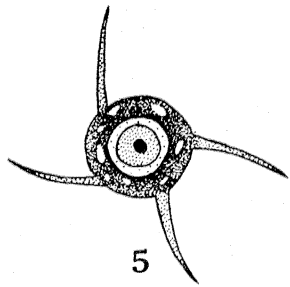

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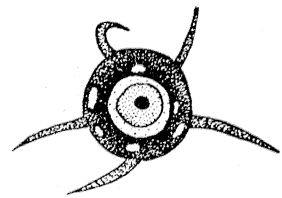

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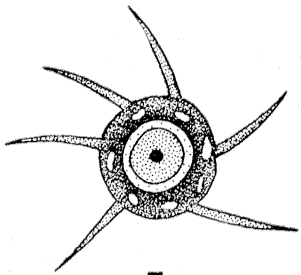

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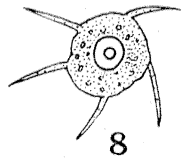

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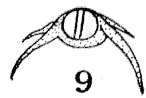

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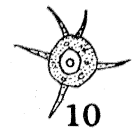

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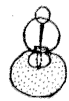

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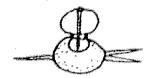

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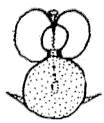

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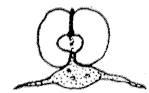

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Explanation of Plate II.

Fig. 24. Spermatozoön which has exploded in a hypotonic solution of calcium chloride.

Figs. 25 to 28. Spermatozoa which have been exposed to various concentrations of potassium chloride.

Figs. 29 to 31. Spermatozoa in weak solutions of potassium hydroxide.

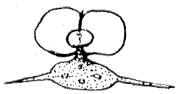
Figs. 32 and 33. Spermatozoa which have exploded in distilled water.

Figs. 34 to 39. Spermatozoa which have been subjected to the effects of ovarian fluids.

Figs. 40 to 43. Spermatozoa which have been acted on by glacial acetic acid in sea water.

Fig. 44. Bottom view of spermatozoön which has been exposed to saponin in sea water.

Figs. 45 and 46. Spermatozoa which have been acted on by nitric acid in sea water.



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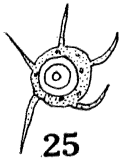

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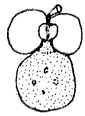

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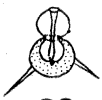

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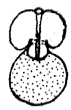

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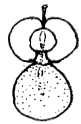

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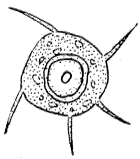

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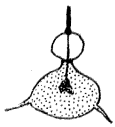

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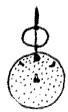

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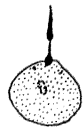

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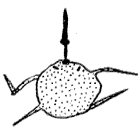

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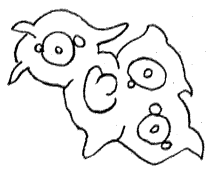

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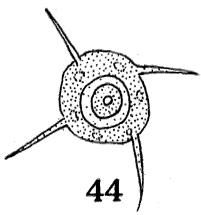

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